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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/758,007	01/10/2001	Leonard I. Zon	701039-50920	8096

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EXAMINER	
LI, QIAN JANICE	
ART UNIT	**PAPER NUMBER**
1632	

DATE MAILED: 07/12/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/758,007	ZON ET AL.	
	Examiner	Art Unit	
	Q. Janice Li	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 May 2004* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-29* is/are pending in the application.

4a) Of the above claim(s) *1-17* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *18-29* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *11 June 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5/4/04 has been entered.

The amendment and remarks filed 1/20/04 have been entered. Claims 1-29 are pending, claim 18 has been amended, claims 1-17 have been withdrawn from further consideration by the Examiner, pursuant to 37 CFR 1.142(b), as being drawn to non-elected inventions. Claims 18-29 are under current examination.

Unless otherwise indicated, previous rejections that have been rendered moot in view of the amendment to pending claims will not be reiterated. The arguments in 1/20/04 response would be addressed to the extent that they apply to current rejection.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 18-29 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 18, 19, 21-29 stand rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted step is: how a mutant gene is identified so that the body of the claim would clearly relate back to the preamble. The amendment of claim 18 fails to obviate this rejection because the steps of claim 18 would result in the identification of a fish that harbors a gene mutation associated with tumor formation, but they would not pinpoint to which gene mutation that causes or accelerates the tumor formation. Thus, the essential step of identifying a gene mutation is still missing from the method steps of the claims.

Claim 18 recites the limitation, "the mutant fish" in step g. There is insufficient antecedent basis for this limitation in the claim. Further, there is a mutant fish in almost every step of claim 18, it is unclear which fish the term "mutant fish" refers to, thus the metes and bounds of the claims are uncertain.

Claim 18 recites, "exposing eggs of said F1 generation", which encompasses both a fertilized or un-fertilized egg. It is unclear whether the claim encompasses both, and how an unfertilized egg could generate a haploid embryo with an inactivated sperm, and thus the metes and bounds of the claims are uncertain.

Claims are vague and indefinite because of claim (18) recitation "mutagen" in step a, and "carcinogen" in step f. Since a chemical may act both as a mutagen and a

carcinogen, it is unclear whether the recited mutagen and carcinogen refer to the same or two different agents in step a and step f, thus the metes and bounds of the claims are unclear.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Previous rejections under this section have been modified to address the amendments and arguments.

Claims 18 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Cheng et al* (Biochem Cell Biol 1997;75:525-533), in view of *Spitsbergen et al*

(Toxicol Pathol 2000;28:716-725, IDS/CH), and evidenced by *Couch et al* (Toxicol Pathol 1996;24:602, IDS).

These claims are drawn to a method comprising screening haploid embryo of F1 and the tumor formation of F2 zebrafish for identifying a gene mutation involved in carcinogenesis.

Cheng et al teach methods of genetic dissection in zebrafish for *identify genes* in biological processes for discovery of different types of *mutations* (See e.g. abstract and last section in page 532), which are particularly useful for assaying *recessive gene mutations* (see e.g. the title and the first sentence of the abstract). For uniparental and two-generation screens. *Cheng et al* outlined steps necessary in figure 1, which start with "1. mutagenesis" (equivalent of instant step a, exposing a fish to a mutagen) and "2. outcross" with a wild-type fish to produce an F1 generation (instant step b); followed by exposing the fertilized eggs of F1 generation to UV-inactivated sperm creating haploid embryos (instant step c) for the haploid screen and half-tetrad screen methods. They go on to teach, "FOR EACH F2 PARENT IN A RANDOM CROSS, THERE IS A ½ CHANCE OF CARRYING THE MUTATION. THEREFORE, BOTH PARENTS WILL CARRY THE MUTATION IN ONLY ¼ OF RANDOM CROSSES. FOR THIS REASON, *MULTIPLE CROSSES* ARE ATTEMPTED PER FAMILY" ((last paragraph of page 528, instant steps (b), (c), (d), (e)). For the mechanism of outcrossing, *Cheng et al* teach, "IN GYNOGENETIC SCREENS, MENDELIAN INHERITANCE CAN BE PROVEN BY OUTCROSSING F1 FEMALES THAT ARE KNOWN CARRIERS OF INTERESTING MUTATIONS, AND THEN IDENTIFYING THE MUTATIONS IN THE F2 BY CROSSES OF SIBLINGS OR A SECOND ROUND OF HALF-TETRAD ANALYSIS. (right column, page 530, step d). *Chang et al* compared the characteristics of the three genetic dissection methods (table 2) and teach "THIS

INFORMATION CAN BE USED TO DETERMINE WHAT TYPE OF SCREEN IS MOST APPROPRIATE TO A DESIRED *MUTANT PHENOTYPE*" (left column, page 531). With respect to the potential applicability of such methods, *Cheng et al* teach, "THE INCREASINGLY POWERFUL GENETIC AND EXPERIMENTAL TOOLS AVAILABLE FOR WORK WITH ZEBRAFISH CAN BE USED TO ADDRESS A BROAD RANGE OF QUESTIONS IN VERTEBRATE BIOLOGY" (abstract), and "IT IS ALSO IMPORTANT TO POINT OUT THAT THE EXPERIMENTAL FEATURES THAT MAKE THE ZEBRAFISH SO USEFUL IN DISSECTING THE MYSTERIES OF THE DEVELOPMENT CAN BE APPLIED TO THE ELUCIDATION OF OTHER SIGNIFICANT PROBLEMS IN VERTEBRATE BIOLOGY" (last sentence of the article). Thus, the teaching of *Cheng et al* establishes at the time of filing, the state of art regarding mutant gene discovery and how to make a mutant fish that is suitable for recessive mutant gene recovery *Cheng et al* differs from the instantly claimed invention in that they did not specify what the broad range of questions or significant problems in vertebrate biology encompass, or what particular phenotype to screen for, e.g. screening for cell proliferation defects or tumor formation.

Spitsbergen et al supplemented the teachings of *Cheng et al* by disclosing what phenotype to screen for when studying carcinogenesis, and by illustrating the state of the art regarding experimental fish models in cancer research, which evidenced that cancer research is a significant problem in vertebrate biology and broad range of questions in this area could be addressed by using fish as both a model system and an indicator species. S*pitsbergen et al* teach investigating the carcinogenesis of certain chemical mutagen in zebrafish, comprising exposing the fish to different dose of a carcinogen with different exposure routes (step f) and screening for cell proliferation

defects, e.g. body weight and neoplasia (tumor formation) in zebrafish embryos, fry, and juvenile (e.g. tables 1-3, step d & g).

Although not relied upon, *Crouch et al* further evidenced that the reasonable skilled artisans have used the fish model in cancer research for more than three decades, which establishes that it is well known in the art that carcinogenesis is a significant and persistent problem in vertebrate biology.

The teaching of *Spitsbergen et al* differs from the instantly claimed invention in that they did not use a mutant fish for screening. However, using a mutant fish as a genetic dissection tool and its applicability has been taught by *Cheng et al.* Applicants are reminded that the test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981).

Although none of the above teachings specify compare a mutant fish with a wild-type fish for tumor formation screening, it is a common knowledge in the experimental biology that a proper control is needed as a baseline for comparison in any experiment. Given the knowledge of the skilled in the art, this limitation falls within the bound of optimization as to what kind of control should be used. For example, *Cheng et al* teach when mapping mutations in half-tetrads screening, two pools of DNA are made and compared: one from multiple *mutant* embryos, and the other from multiple *wild-type*

embyos (2nd paragraph, page 532). Here, it evidences that it is a common knowledge in the art to compare the mutant with the wild type for gene mutation study.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to apply the method as taught by *Cheng et al* in cancer research as taught by *Spitsbergen et al* for identifying a recessive gene mutation associated with tumor formation with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to do so because an experimental mutant fish is induced by exposing it to a mutagen, and "MOST INDUCED MUTATIONS ARE RECESSIVE...IT IS THEREFORE ESSENTIAL TO RENDER RECESSIVE MUTATIONS HOMOZYGOUS OR HEMIZYGOUS TO DETECT THERIR PHENOTYPE" (*Cheng et al*, right column, page 527), and this can be achieved by using the methods disclosed by *Chang et al*. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 20, 21, 23, and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Cheng et al* (Biochem Cell Biol 1997;75:525-533), *Spitsbergen et al* (Toxicol Pathol 2000;28:716-725, IDS/CH) as applied to claims 18 and 19 above, further in view of *Driever et al* (J Clin Invest 1996;97:1788-94), and *Alexander et al* (Dev Genet 1998;22:288-299).

These claims are drawn to various means for screening a gene mutation and using irradiation as means of mutation. The combined teachings of *Cheng et al* and *Spitsbergen et al* do not specify these means. However, before the effective filing date, *Driever and Fishman* teach the art known technologies for zebrafish screening, wherein

the first step was identifying efficient conditions for mutagenesis, e.g. ENU (single gene mutations) and irradiation (multigenic lesions), followed by identifying the mutant phenotypes with visual inspection under the microscope (steps d & g, see page 1789). They further teach, the screening based on the visually evident phenotype is insufficient for identifying mutations in organs buried deep within the animal, thus, positional cloning as well as biochemical methods (antibodies and nucleic acid probes) could be used for identifying the mutations (see last section, page 1792). *Driever et al* go on to teach using gene probe and cell population assays in zebrafish screening (§Future directions). *Alexander et al* teach that examining (screening) the molecular markers in the haploid progeny of mosaic F1 females would expedite the screening process (page 293-294) and *Alexander et al* use in situ hybridization (probes) and immunofluorescence for fish embryo screening. The teachings of *Driever et al* and *Alexander et al* illustrated the recited means are known in the art for use in screening for gene mutation.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to apply the means as taught by *Driever et al* and *Alexander et al* in the screening method as taught by *Cheng et al* and *Spitsbergen et al* for identifying a recessive gene mutation associated with a tumor formation with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to do so because given the numerous screening and mutagenic means known in the art, these limitations fall within the bounds of optimization as to which means should be used for making the mutant fish and screening the mutant gene of

interest. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 22 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Cheng et al* (Biochem Cell Biol 1997;75:525-533), *Spitsbergen et al* (Toxicol Pathol 2000;28:716-725, IDS/CH), *Driever et al* (J Clin Invest 1996;97:1788-94), and *Alexander et al* (Dev Genet 1998;22:288-299) as applied to claims 18-21, 23, 29 above, and further in view of *Epstein et al* (US 5,756,476).

Claims 22 and 24 are drawn to protein or gene markers that could be used in haploid embryo screening for cell proliferation defects.

Cheng et al, *Spitsbergen et al, Driever et al,* and *Alexander et al* teach screening haploid embryo by a marker, but do not point out the particular markers recited in the claims. However, before the effective filing date of the instant application, the recited markers, such as PCNA and cyclin-b1, and their association with cell proliferation and tumor formation are known in the art and have been used for diagnosis as taught by *Epstein et al* (see abstract, column 2, lines 28-39).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to apply the PCNA and/or cyclin-b1 markers as taught by *Epstein et al* in haploid embryo screening as taught by *Cheng et al*, *Spitsbergen et al, Driever et al,* and *Alexander et al* for identifying a recessive gene mutation associated with a tumor formation with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to do so because given the numerous markers

known in the art, these limitations fall within the bounds of optimization as to which markers should be used for screening the mutant gene of interest. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claim 25 is rejected under 35 U.S.C. 103(a) as being unpatentable over *Cheng et al* (Biochem Cell Biol 1997;75:525-533), *Spitsbergen et al* (Toxicol Pathol 2000;28:716-725, IDS/CH) as applied to claims 18 and 19 above, and further in view of *Vogelstein et al* (US 6,511,818).

Claim 25 is specifically directed to using flow cytometry detecting dye staining for DNA content indicating a problem in cell proliferation.

The combined teachings of *Cheng et al* and *Spitsbergen et al*, do not specify the particular method.

However, before the effective filing date of instant application, *Vogelstein et al* teach that DNA accumulation occurs in defective checkpoint gene during tumor development, and such change could be detected using flow cytometry assay for DNA content, and could be used for cancer causative agent and drug screening (see particularly, column 1, lines 37-46, and column 3, lines 24-45).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the methods taught by *Cheng et al* and *Spitsbergen et al* with the method taught by *Vogelstein et al,* using DNA content flow cytometry in addition to tumor formation for haploid embryo screening with a reasonable

expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention because the method could detect early changes in tumor formation. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 26 and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Cheng et al* (Biochem Cell Biol 1997;75:525-533) and *Spitsbergen et al* (Toxicol Pathol 2000;28:716-725, IDS/CH) as applied to claims 18 and 19 above, and further in view of *O'Reilly et al* (US 5,854,205).

Claims 26 and 27 are drawn to using apoptosis markers such as TUNEL stain in haploid embryo screening for cell proliferation defects.

Cheng et al and *Spitsbergen et al* teach screening haploid embryo, but do not point out the particular means of screening recited in the claims. However, before the effective filing date of the instant application, the recited means, e.g. TUNEL stain, and their association with cell proliferation and tumor formation are known in the art, as taught by *O'Reilly et al* (see figs. 8, 12, column 6, lines 12-24).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the methods taught by *Cheng et al* and *Spitsbergen et al* with the method taught by *O'Reilly et al* using TUNEL stain for haploid embryo screening with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention because the method could further confirm/early detect cell proliferation defect related to cancer. Thus, the claimed

invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claim 28 is rejected under 35 U.S.C. 103(a) as being unpatentable over *Cheng et al* (Biochem Cell Biol 1997;75:525-533) and *Spitsbergen et al* (Toxicol Pathol 2000;28:716-725, IDS/CH), as applied to claims 18 and 19 above, further in view of *Li et al* (US 5,679,523).

Claim 28 is drawn to using BrdU staining in haploid embryo screening for cell proliferation defects.

Cheng et al and *Spitsbergen et al* teach screening haploid embryo, but do not specify a particular means. However, before the effective filing date of the instant application, BrdU as a marker for fast diagnosis of tumor is known in the art, as taught by *Li et al* (column 10, lines 10-15).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the methods taught by *Cheng et al* and *Spitsbergen et al* with the method as taught by *Li et al* using BrdU for haploid embryo screening with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention because given the numerous markers known in the art for assessing cell proliferation defect related to cancer, this limitation falls within the bounds of optimization as to which means is used for screening. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Q. Janice Li** whose telephone number is 571-272-0730. The examiner can normally be reached on 9:30 am - 7 p.m., Monday through Friday, except every other Wednesday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Amy Nelson** can be reached on 571-272-0804. The fax numbers for the organization where this application or proceeding is assigned are **703-872-9306**.

Any inquiry of formal matters can be directed to the patent analyst, **Dianiece Jacobs**, whose telephone number is (571) 272-0532.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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JANICE LI
PATENT EXAMINER

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QL
July 9, 2004